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(FILE 'HOME' ENTERED AT 08:41:54 ON 21 NOV 2002)

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SEA FUCOSYLTRANSFERASE

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L1 QUE FUCOSYLTRANSFERASE

FILE 'CAPLUS, BIOSIS, MEDLINE, SCISEARCH, EMBASE, BIOTECHNO, ESBIODBASE, CANCERLIT' ENTERED AT 08:43:19 ON 21 NOV 2002

L2 1355 S L1 AND (LEWIS X)

L3 100 S L2 AND HELICOBACTER

L4 40 S L3 AND (ISOLAT? OR PURIF? OR CDNA OR CLONE)

L5

15 DUP REM L4 (25 DUPLICATES REMOVED)

=> d 15 ibib ab 10-15

L5 ANSWER 10 OF 15 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 1998:746829 SCISEARCH

THE GENUINE ARTICLE: 122RN

TITLE: Human alpha 1,3/4-**fucosyltransferases** - III. A
Lys/Arg residue located within the alpha 1,3-FucT motif is
required for activity but not substrate binding

AUTHOR: Sherwood A L; Nguyen A T; Whitaker J M; Macher B A; Stroud
M R; Holmes E H (Reprint)

CORPORATE SOURCE: NW HOSP, PACIFIC NW CANC FDN, DIV CELL SURFACE BIOCHEM,
120 NORTHGATE PLAZA, SUITE 218, SEATTLE, WA 98125
(Reprint); NW HOSP, PACIFIC NW CANC FDN, DIV CELL SURFACE
BIOCHEM, SEATTLE, WA 98125; SAN FRANCISCO STATE UNIV, DEPT
CHEM & BIOCHEM, SAN FRANCISCO, CA 94132

COUNTRY OF AUTHOR: USA

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (25 SEP 1998) Vol. 273,
No. 39, pp. 25256-25260.

Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC,
9650 ROCKVILLE PIKE, BETHESDA, MD 20814.

ISSN: 0021-9258.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 36

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Amino acid sequence alignment of human alpha 1,3/4-
fucosyltransferases (FucTs) demonstrates that three highly
conserved Lys residues are present in the catalytic domain of FucTs III,
TV, V, and VI. Two of these sites are conserved in FucT VII, with the
third located within the alpha 1,3-FucT motif as a conservative change to
Arg at position 223. Site-directed mutagenesis experiments were conducted
to change Lys(255) of FucT V (equivalent to Arg(223) of FucT VII) to
either Arg(255) or Ala(255). Enzyme assays demonstrate that the FucT V
K255R mutant has a 34-fold lower specific activity than native FucT V and
that the K255A mutant is inactive. Site-directed mutagenesis of FucT VII
was also conducted to change Arg(223) to Lys(223) for analysis of the
effect on enzyme kinetic parameters. No differences in acceptor
specificities or K-m values for either substrate were observed between
native FucT VII and the R223K mutant; however, the **purified**
R223K mutant enzyme had a 2-fold increased specific activity compared with
purified native FucT VII. No change in GDP-fucose-protectable
pyridoxal-P/NaBH4 inactivation was observed for native or mutant FucT V or
VII, further supporting the absence of involvement of this residue in
sugar nucleotide binding. The results indicate that a basic residue in
this position is required for enzyme activity, with a Lys residue
providing higher intrinsic activity. The lack of influence of this site on
substrate binding parameters and its location within the alpha 1,3-FucT
motif suggest that at least some of the residues within this motif are
involved in catalysis rather than substrate binding.

L5 ANSWER 11 OF 15 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 1998:642782 SCISEARCH

THE GENUINE ARTICLE: 111MB

TITLE: Molecular cloning and characterization of an alpha 1,3
fucosyltransferase, CEFT-1, from *Caenorhabditis*
elegans

AUTHOR: DeBoseBoyd R A; Nyame A K; Cummings R D (Reprint)

CORPORATE SOURCE: UNIV OKLAHOMA, HLTH SCI CTR, DEPT BIOCHEM & MOL BIOL, BRC
417, 975 NE 10TH ST, OKLAHOMA CITY, OK 73104 (Reprint);
UNIV OKLAHOMA, HLTH SCI CTR, DEPT BIOCHEM & MOL BIOL,
OKLAHOMA CITY, OK 73104

COUNTRY OF AUTHOR: USA

SOURCE: GLYCOBIOLOGY, (SEP 1998) Vol. 8, No. 9, pp. 905-917.

Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD
OX2 6DP, ENGLAND.

ISSN: 0959-6658.

DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 54

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We report on the identification, molecular cloning, and characterization of an alpha 1,3 **fucosyltransferase** (alpha 1,3FT) expressed by the nematode, *Caenorhabditis elegans*. Although *C. elegans* glycoconjugates do not express the **Lewis x** antigen Gal beta 1-->4[Fuc alpha 1-->3]GlcNAc beta-->R, detergent extracts of adult *C. elegans* contain an alpha 1,3FT that can fucosylate both nonsialylated and sialylated acceptor glycans to generate the Le(X) and sialyl Le(X) antigens, as well as the lacdiNAc-containing acceptor GalNAc beta 1-->4GlcNAc beta 1-->R to generate GalNAc beta 1-->4 [Fuc alpha 1-->3]GlcNAc beta 1-->R. A search of the *C. elegans* genome database revealed the existence of a gene with 20-23% overall identity to all five cloned human alpha 1,3FTs. The putative **cDNA** for the *C. elegans* alpha 1,3FT (CEFT-1) was amplified by PCR from a **cDNA** lambda ZAP library, cloned, and sequenced. COS7 cells transiently transfected with **cDNA** encoding CEFT-1 express the Le(X), but not sLe(X) antigen. The CEFT-1 in the transfected cell extracts can synthesize Le(X), but not sialyl Le(X), using exogenous accepters. A second **fucosyltransferase** activity was detected in extracts of *C. elegans* that transfers Fuc in alpha 1,2 linkage to Gal specifically on type-1 chains. The discovery of alpha-**fucosyltransferases** in *C. elegans* opens the possibility of using this well-characterized nematode as a model system for studying the role of fucosylated glycans in the development and survival of *C. elegans* and possibly other helminths.

L5 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 4

ACCESSION NUMBER: 1998:7807 CAPLUS

DOCUMENT NUMBER: 128:190186

TITLE: Phase variation in **Helicobacter pylori**
lipopolysaccharide

AUTHOR(S): Appelmelk, B. J.; Shiberu, B.; Trinks, C.; Tapsi, N.;
Zheng, P. Y.; Verboom, T.; Maaskant, J.; Hokke, C. H.;
Schiphorst, W. E. C. M.; Blanchard, D.; Simoons-Smit,
I. M.; Van Den Eijnden, D. H.; Vandenbroucke-Grauls,
C. M. J. E.

CORPORATE SOURCE: Department of Medical Microbiology, Medical School,
Vrije Universiteit, Amsterdam, 1081 BT, Neth.

SOURCE: Infection and Immunity (1998), 66(1), 70-76

CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Helicobacter pylori** NCTC 11637 lipopolysaccharide (LPS) expresses the human blood group antigen **Lewis x** (Lex) in a polymeric form. Lex is .beta.-D-galactose-(1-4)-[.alpha.-L-fucose-(1-3)]-.beta.-D-acetylglucosamine. Schematically the LPS structure is (Lex)n-core-lipid A. In this report, we show that Lex expression is not a stable trait but that LPS displays a high frequency (0.2 to 0.5%) of phase variation, resulting in the presence of several LPS variants in one bacterial cell population. One type of phase variation implied the loss of .alpha.1,3-linked fucose, resulting in variants that expressed nonsubstituted poly lactosamines (also called the i antigen), i.e., Lex minus fucose; LPS: (lactosamine)n-core-lipid A. The switch of Lex to i antigen was reversible. A second group of variants arose by loss of polymeric main chain which resulted in expression of monomeric Ley; LPS: (Ley)-core-lipid A. A third group of variants arose by acquisition of .alpha.1,2-linked fucose which hence expressed Lex plus Ley; LPS: (Ley)

(Lex)n-core-lipid A. The second and third group of variants switched back to the parental phenotype [(Lex)n-core-lipid A] in lower frequencies. Part of the variation can be ascribed to altered expression levels of glycosyltransferase levels as assessed by assaying the activities of galactosyl-, fucosyl-, and N-acetylglucosaminyltransferases. Clearly phase variation increases the heterogeneity of *H. pylori*, and this process may be involved in generating the very closely related yet genetically slightly different strains that have been isolated from one patient.

L5 ANSWER 13 OF 15 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 5

ACCESSION NUMBER: 97268464 EMBASE

DOCUMENT NUMBER: 1997268464

TITLE: Cloning and heterologous expression of an α .1,3-fucosyltransferase gene from the gastric pathogen *Helicobacter pylori*.

AUTHOR: Ge Z.; Chan N.W.C.; Palcic M.M.; Taylor D.E.

CORPORATE SOURCE: D.E. Taylor, Medical Microbiol./Immunology Dept., 1-28 Medical Sciences Bldg., University of Alberta, Edmonton, Alta. T6G 2H7, Canada. diane.taylor@ualberta.ca

SOURCE: Journal of Biological Chemistry, (1997) 272/34 (21357-21363).

Refs: 56

ISSN: 0021-9258 CODEN: JBCHA3

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB *Helicobacter pylori* is an important human pathogen which causes both gastric and duodenal ulcers and is also associated with gastric cancer and lymphoma. This microorganism has been shown to express cell surface glycoconjugates including Lewis X (Le(X)) and Lewis Y. These bacterial oligosaccharides are structurally similar to tumor-associated carbohydrate antigens found in mammals. In this study, we report the cloning of a novel α .1,3-fucosyltransferase gene (HpFucT) involved in the biosynthesis of Le(X) within *H. pylori*. The deduced amine acid sequence of HpFucT consists of 478 residues with the calculated molecular mass of 56,194 daltons, which is approximately 100 amino acids longer than known mammalian α .1,3/1,4-fucosyltransferases. The approx.52-kDa protein encoded by HpFucT was expressed in *Escherichia coli* CSRDE3 cells and gave rise to α .1,3-fucosyltransferase activity but neither α .1,4-fucosyltransferase nor α .1,2-fucosyltransferase activity as characterized by radiochemical assays and capillary zone electrophoresis. Truncation of the C-terminal 100 amino acids of HpFuc-T abolished the enzyme activity. An approximately 72-amino acid region of HpFuc-T exhibits significant sequence identity (40-45%) with the highly conserved C-terminal catalytic domain among known mammalian and chicken α .1,3-fucosyltransferases. These lines of evidence indicate that the HpFuc-T represents the bacterial α .1,3-fucosyltransferase. In addition, several structural features unique to HpFuc-T, including 10 direct repeats of seven amine acids and the lack of the transmembrane segment typical for known eukaryotic α .1,3-fucosyltransferases, were revealed. Notably, the repeat region contains a leucine zipper motif previously demonstrated to be responsible for dimerization of various basic region-leucine zipper proteins, suggesting that the HpFuc-T protein could form dimers.

L5 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 6

ACCESSION NUMBER: 1997:567080 CAPLUS

DOCUMENT NUMBER: 127:258343

TITLE: Lewis X biosynthesis in *Helicobacter pylori*. Molecular cloning of an

.alpha.(1,3)-**fucosyltransferase** gene
 AUTHOR(S): Martin, Stephen L.; Edbrooke, Mark R.; Hodgman, T. Charles; Van Den Eijnden, Dirk H.; Bird, Michael I.
 CORPORATE SOURCE: Glycobiology Unit, Glaxo Wellcome Medicines Research Centre, Stevenage, SG1 2NY, UK
 SOURCE: Journal of Biological Chemistry (1997), 272(34), 21349-21356
 CODEN: JBCHA3; ISSN: 0021-9258
 PUBLISHER: American Society for Biochemistry and Molecular Biology
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB The lipopolysaccharide of certain strains of **Helicobacter pylori** was recently shown to contain the **Lewis X** (Lex) trisaccharide (Gal.beta.-1,4-(Fuc.alpha.(1,3))-GlcNAc). Lex is an oncofetal antigen which appears on human gastric epithelium, and its mimicry by carbohydrate structures on the surface of *H. pylori* may play an important part in the interaction of this pathogen with its host. Potential roles for bacterial Lex in mucosal adhesion, immune evasion, and autoantibody induction have been proposed (Moran, A. P., Prendergast, M. M., and Appelmelk, B. J. (1996) FEMS Immunol. Med. Microbiol. 16, 105-115). In mammals, the final step of Lex biosynthesis is the .alpha.(1,3)-fucosylation of GlcNAc in a terminal Gal.beta.(1.fwdarw.4)-GlcNAc unit, and a corresponding GDP-fucose:N-acetyl-glucosaminyl .alpha.(1,3) **fucosyltransferase** (.alpha.(1,3)-Fuc-T) activity was recently discovered in *H. pylori* exts. Part of a human .alpha.(1,3)-Fuc-T amino acid sequence was used to search an *H. pylori* genomic data base for related sequences. Using a probe based upon weakly matching data base sequences, **clones** from a plasmid library of *H. pylori* DNA were retrieved. DNA sequence anal. of the library **clones** revealed a gene which was named fucT, encoding a protein with localized homol. to the human .alpha.(1,3)-Fuc-Ts. It has been demonstrated that fucT encodes an active Fuc-T enzyme by expressing the gene in *Escherichia coli*. The recombinant enzyme shows a strong preference for type 2 (e.g. LacNAc) over type 1 (e.g. lacto-N-biose) acceptors in vitro. Certain residues in a short segment of the *H. pylori* protein are completely conserved throughout the .alpha.(1,3)-Fuc-T family, defining an .alpha.(1,3)-Fuc-T motif which may be of use in identifying new **fucosyltransferase** genes.

L5 ANSWER 15 OF 15 MEDLINE DUPLICATE 7
 ACCESSION NUMBER: 96195860 MEDLINE
 DOCUMENT NUMBER: 96195860 PubMed ID: 8608270
 TITLE: The biosynthesis of **Lewis X** in **Helicobacter pylori**.
 AUTHOR: Chan N W; Stangier K; Sherburne R; Taylor D E; Zhang Y; Dovichi N J; Palcic M M
 CORPORATE SOURCE: Department of Chemistry, University of Alberta, Edmonton, Canada.
 SOURCE: GLYCOBIOLOGY, (1995 Oct) 5 (7) 683-8.
 Journal code: 9104124. ISSN: 0959-6658.
 PUB. COUNTRY: ENGLAND: United Kingdom
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199605
 ENTRY DATE: Entered STN: 19960605
 Last Updated on STN: 19980206
 Entered Medline: 19960530

AB The biosynthesis of the **Lewis X** determinant (Gal beta 1-4 [Fuc alpha 1-3]GlcNAc beta-) in three strains of **Helicobacter pylori** has been investigated. Strains UA 861, UA 802 and UA 1182 contain alpha 1,3 **fucosyltransferase** and beta 1,4 galactosyltransferase activities that synthesize the **Lewis X** structure by

the transfer of monosaccharides from GDP-fucose and UDP-galactose donors, respectively. The enzyme reaction products that formed were characterized by capillary zone electrophoresis and by ¹H-NMR spectroscopy. The biosynthetic pathway is therefore identical to that found in humans. In the three strains, the **fucosyltransferase** and galactosyltransferase activities differed in various cellular fractions. K_m values for their donor and acceptor substrates also differed.

=> d 15 ibib ab 1-9

L5 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:314468 CAPLUS

DOCUMENT NUMBER: 136:324173

TITLE: Chimeric genes encoding enzymes for biosynthesis of GDP-L-fucose and fucosylated glycans from GDP-D-mannose for treatment of infections and inflammation

INVENTOR(S): Renkonen, Risto; Mattila, Pirkko; Hirvas, Laura; Hortling, Solveig; Kallioinen, Tuula; Kauranen, Sirkka-liisa; Jaervinen, Nina; Maeki, Minna; Niittymaeki, Jaana; Raebinae, Jarkko

PATENT ASSIGNEE(S): Medicel Oy, Finland

SOURCE: Eur. Pat. Appl., 28 pp.

CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| EP 1199364 | A2 | 20020424 | EP 2001-660180 | 20010925 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR | | | | |
| FI 2000002114 | A | 20020327 | FI 2000-2114 | 20000926 |
| US 2002058313 | A1 | 20020516 | US 2001-962805 | 20010926 |

PRIORITY APPLN. INFO.: FI 2000-2114 A 20000926

AB Use of recombinant enzymes for the prepn. of GDP-L-fucose and fucosylated glycans is disclosed. GDP-L-fucose functions as a fucose donor in the biosynthetic route leading to the fucosylated glycans, which have therapeutic utility. A process for prepg. GDP-L-fucose and fucosylated glycans, and means useful in the process are provided. Said means include enzymes, chimeric enzymes, DNA sequences, genes, vectors and host cells. Fucosylation of glycans on glycoproteins and -lipids requires the enzymic activity of relevant **fucosyltransferases** and GDP-L-fucose as the donor. Due to the biol. importance of fucosylated glycans, a readily accessible source of GDP-L-fucose would be required. Here the authors describe the construction of a stable recombinant *S.cerevisiae* strain expressing the *E.coli* genes *gmd* and *wcaG* encoding the two enzymes, GDP-mannose-4,6-dehydratase (GMD) and GDP-4-keto-6-deoxy-D-mannose-3,5-epimerase/4-reductase (GFS) resp., needed to convert GDP-mannose to GDP-fucose via the de novo pathway. Taking advantage of the rich inherent cytosolic GDP-mannose pool in *S.cerevisiae* cells the authors produced 0.2 mg/l of GDP-L-fucose with the recombinant yeast strain without addn. of any external GDP-mannose. The GDP-L-fucose product may be used as the fucose donor for α -1,3-**fucosyltransferase** to synthesize sialyl **Lewis x** (sLex), a glycan crucial for the selectin-dependent leukocyte traffic. GDP-L-fucose may also be prepd. using the salvage pathway from L-fucose by fucokinase (FK) and GDP-fucose-pyrophosphorylase (PP), synthesized from a chimeric gene. Two rapid and simple procedures for the quant. anal. of GDP-L-fucose (GDP-Fuc) are described. The methods are based on time-resolved fluorescence and microplate assay technol. The first assay relies on measuring the enzyme activity of α -1,3-**fucosyltransferase**. In this assay, transfer of fucose from GDP-Fuc converts sialyllactosamine to sialyl **Lewis x** tetrasaccharide, which is detected and quantified by relevant antibodies on a microplate. The formation of the reaction product is directly dependent on the presence of GDP-Fuc in the concn. range of 10-10,000 nM. In the second method GDP-Fuc inhibits the binding of fucose-specific *Aleuria aurantia* lectin to fucosylated glycan on a microwell. The lectin-based assay is less sensitive than the enzyme

assay, but it is cheaper and faster. The authors used these assays in monitoring the amt. of GDP-Fuc in crude lysates of transgenic yeast, which expresses the enzymes producing GDP-Fuc. The newly developed assays are versatile and applicable to measure also other nucleotide sugars or glycosyltransferase activities in a high-throughput manner.

L5 ANSWER 2 OF 15 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 1

ACCESSION NUMBER: 2002191218 EMBASE

TITLE: **Helicobacter** priori does not require
Lewis X or Lewis Y expression to colonize
C3H/HeJ mice.

AUTHOR: Takata T.; El-Omar E.; Camorlinga M.; Thompson S.A.;
Minohara Y.; Ernst P.B.; Blaser M.J.

CORPORATE SOURCE: T. Takata, First Dept. of Internal Medicine, School of
Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-Ku,
Fukuoka 814-0180, Japan. takatt01@med.nyu.edu

SOURCE: Infection and Immunity, (2002) 70/6 (3073-3079).
Refs: 39

ISSN: 0019-9567 CODEN: INFIBR

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
048 Gastroenterology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB **Helicobacter** pylori strains frequently express **Lewis**
X (Le(x)) and/or Le(y) on their cell surfaces as constituents of
the O antigens of their lipopolysaccharide molecules. To assess the effect
of Le(x) and Le(y) expression on the ability of *H. pylori* to colonize the
mouse stomach and to adhere to epithelial cells, isogenic mutants were
created in which fucT1 alone or fucT1 and fucT2, which encode the fucosyl
transferases necessary for Le(x) and Le(y) expression, were deleted.
C3H/HeJ mice were experimentally challenged with either wild-type 26695 *H.*
pylori or its isogenic mutants. All strains, whether passaged in the
laboratory or recovered after mouse passage, colonized the mice well and
without consistent differences. During colonization by the mutants, there
was no reversion to wild type. Similarly, adherence to AGS and KatoIII
cells was unaffected by the mutations. Together, these findings indicate
that Le expression is not necessary for mouse gastric colonization or for
H. pylori adherence to epithelial cells.

L5 ANSWER 3 OF 15 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 2001:478310 SCISEARCH

THE GENUINE ARTICLE: 441AY

TITLE: Heterologous over-expression of alpha-1,6-
fucosyltransferase from *Rhizobium* sp.: Application
to the synthesis of the trisaccharide beta-D-GlcNAc(1 ->
4)-[alpha-L-Fuc-(1 -> 6)]-D-GlcNAc, study of the acceptor
specificity and evaluation of polyhydroxylated
indolizidines as inhibitors

AUTHOR: Bastida A; Fernandez-Mayoralas A; Arrayas R G; Iradier F;
Carretero J C; Garcia-Junceda E (Reprint)

CORPORATE SOURCE: CSIC, Inst Quim Organ Gen, Dept Quim Organ Biol, Juan
Cierva 3, E-28006 Madrid, Spain (Reprint); CSIC, Inst Quim
Organ Gen, Dept Quim Organ Biol, E-28006 Madrid, Spain;
Univ Autonoma Madrid, Fac Ciencias, Dept Quim Organ,
E-28049 Madrid, Spain

COUNTRY OF AUTHOR: Spain

SOURCE: CHEMISTRY-A EUROPEAN JOURNAL, (1 JUN 2001) Vol. 7, No. 11,
pp. 2390-2397.
Publisher: WILEY-V C H VERLAG GMBH, PO BOX 10 11 61,
D-69451 BERLIN, GERMANY.
ISSN: 0947-6539.

DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 78

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An efficient heterologous expression system for overproduction of the enzyme α -1,6-Fucosyltransferase (α -1,6-FucT) from *Rhizobium* sp, has been developed. The gene coding for the α -1,6-FucT was amplified by PCR using specific primers. After purification, the gene was cloned in the plasmid pKK2233. The resulting plasmid, pKK1.6FucT, was transformed into the *E. coli* strain XL1-Blue MRF'. The protein was expressed both as inclusion bodies and in soluble form. Changing the induction time a five-fold increase of enzyme expressed in soluble form was obtained. In this way five units of enzyme α -1,6-FucT can be obtained per liter of culture. A crude preparation of the recombinant enzyme was used for the synthesis of the branched trisaccharide α -D-GlcNAc-(1 \rightarrow 4)-[α -L-Fuc-(1 \rightarrow 6)]-D-GlcNAc (3), from chitobiose (2) and GDP-Fucose (1). After purification, the trisaccharide 3 was obtained in a 84% overall yield. In order to elucidate the structural requirements for the acceptors, the specificity of the enzyme was studied towards mono-, di- and trisaccharides, which are structurally related to chitobiose. The enzyme uses, among others, the disaccharide N-acetyl lactosamine as a good substrate: the monosaccharide GlcNAc is a weak acceptor. Finally, several racemic polyhydroxylated indolizidines have been tested as potential inhibitors of the enzyme. Indolizidine 21 was the best inhibitor with an IC_{50} of 4.5×10^{-5} M. Interestingly, this compound turned out to be the best mimic for the structural features of the fucose moiety in the presumed transition state.

L5 ANSWER 4 OF 15 MEDLINE

ACCESSION NUMBER: 2002341969 MEDLINE
DOCUMENT NUMBER: 22079564 PubMed ID: 12084982
TITLE: In vivo fucosylation of lacto-N-neotetraose and lacto-N-neohexaose by heterologous expression of *Helicobacter pylori* α -1,3 fucosyltransferase in engineered *Escherichia coli*.
AUTHOR: Dumon C; Priem B; Martin S L; Heyraud A; Bosso C; Samain E
CORPORATE SOURCE: Centre de Recherches sur les Macromolécules Vegetales, 601 rue de la Chimie, BP53X, 38041 Grenoble cedex 09, France.
SOURCE: GLYCOCONJUGATE JOURNAL, (2001 Jun) 18 (6) 465-74.
Journal code: 8603310. ISSN: 0282-0080.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200210
ENTRY DATE: Entered STN: 20020627
Last Updated on STN: 20021004
Entered Medline: 20021003

AB We report here the in vivo production of type 2 fucosylated-N-acetyl lactosamine oligosaccharides in *Escherichia coli*. Lacto-N-neofucopentaose Galbeta1-4GlcNAcbeta1-3Galbeta1-4(Fucalpha1-3)Glc, lacto-N-neodifucohexaose Galbeta1-4(Fucalpha1-3)Glc-NACbeta1-3Galbeta1-4(Fucalpha1-3)Glc, and lacto-N-neodifucooctaose Galbeta1-4GlcNAcbeta1-3Galbeta1-4(Fucalpha1-3)GlcNAcbeta1-3Galbeta1-4(Fucalpha1-3)Glc were produced from lactose added in the culture medium. Two of them carry the Lewis X human antigen. High cell density cultivation allowed obtaining several grams of fucosylated oligosaccharides per liter of culture. The fucosylation reaction was catalyzed by an α -1,3 fucosyltransferase of *Helicobacter pylori* overexpressed in *E. coli* with the genes lgtAB of *N. meningitidis*. The strain was genetically engineered in order to provide GDP-fucose to the system, by genomic inactivation of gene wcaJ involved in colanic acid synthesis and overexpression of RcsA, positive regulator of the colanic acid operon. To prevent fucosylation at the glucosyl residue, lactulose Galbeta1-4Fru was

assayed in replacement of lactose. Lactulose-derived oligosaccharides carrying fucose were synthesized and characterized. Fucosylation of the fructosyl residue was observed, indicating a poor acceptor specificity of the **fucosyltransferase** of *H. pylori*.

L5 ANSWER 5 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:201412 BIOSIS
DOCUMENT NUMBER: PREV200200201412
TITLE: The role of redundant alpha-3-**fucosyltransferase** genes futA and futB in Lewis antigen x/y synthesis by **Helicobacter pylori**.
AUTHOR(S): Baker, P. E. (1); Rosenberg, J. B. (1); Mefford, M. E.; Thevenot, T. L.
CORPORATE SOURCE: (1) Ohio State University, Columbus, OH USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 292.
<http://www.asmtusa.org/mtgsrc/generalmeeting.htm>. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001
ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English

AB The gastric pathogen *H. pylori* expresses two alpha-3-**fucosyltransferases**, which may play different roles in the synthesis of Lewis antigens on *H. pylori* lipopolysaccharide (LPS). These enzymes are encoded by futA and futB, which contain poly(C) regions vulnerable to slip-strand mispairing during replication. The purpose of this study was to determine the relationship of futA and futB expression with Lewis antigen phenotype and animal colonization. futA and futB were amplified from lab-passed and animal-passed *H. pylori* strains. The resultant PCR products were sequenced and the length of the poly(C) tract was determined. In addition, antibodies specific for **Lewis x** or **Lewis y** antigens were used to determine the LPS phenotype by western blot and ELISA. In the two animal-virulent strains examined, SS1 and 26695, there was no relationship between futA sequence, **Lewis x** or **y** expression, or animal passage. The length of the poly(C) region of futA varied between strains and between **isolates** of the same strain, but a relationship between the on/off status of the gene and bacterial phenotype could not be established. In contrast, futB expression was more stable. Variations were present in the poly(C) tract, but predicted amino acid sequence revealed that the gene was still expressed. Like futA, futB expression did not correlate with **Lewis x** expression, but because both futB and **Lewis y** were expressed in all strains, no relationship could be determined. When **isolates** of the same strain were compared, there was no relationship between futB expression and animal virulence. However, pig and mouse virulent strains differed from each other. Pig strains expressed both Lewis antigens (regardless of level of virulence), while mouse strains expressed only **Lewis y**. In animal virulent strains of *H. pylori* futB appears to be sufficient for **Lewis x/y** synthesis, but futA is not necessary. Furthermore, neither futA nor **Lewis x** determine level of virulence of an individual strain for its host. The role of **Lewis x** and **y** in determining host species specificity will require further investigation.

L5 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:314847 CAPLUS
DOCUMENT NUMBER: 132:330636
TITLE: Sequences of **Helicobacter pylori** .alpha.1,2-**fucosyltransferase**, and uses thereof in diagnosing disorders and in monitoring diseases
INVENTOR(S): Taylor, Diane E.; Wang, Ge; Palcic, Monica
PATENT ASSIGNEE(S): Governors of the University of Alberta, Can.
SOURCE: PCT Int. Appl., 71 pp.

CODEN: PIXXD2

DOCUMENT TYPE:

Patent

LANGUAGE:

English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------------------|--|----------|-----------------|------------|
| WO 2000026383 | A1 | 20000511 | WO 1999-CA1031 | 19991103 |
| W: | AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | |
| RW: | GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | |
| US 6238894 | B1 | 20010529 | US 1999-433598 | 19991102 |
| EP 1127138 | A1 | 20010829 | EP 1999-953470 | 19991103 |
| R: | AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | |
| JP 2002528122 | T2 | 20020903 | JP 2000-579755 | 19991103 |
| US 2002037570 | A1 | 20020328 | US 2001-848838 | 20010503 |
| PRIORITY APPLN. INFO.: | | | US 1998-107268P | P 19981104 |
| | | | US 1999-433598 | A 19991102 |
| | | | WO 1999-CA1031 | W 19991103 |

OTHER SOURCE(S): MARPAT 132:330636

AB This invention provides protein and DNA sequences for a newly identified **Helicobacter pylori** .alpha.1,2-fucosyltransferase, which is involved in biosynthesis of fucosylated oligosaccharides including **Lewis X**, Lewis Y, Lewis B and H type 1, which are structurally similar to certain tumor-assocd. carbohydrate antigens found in mammals. The center region of fuct2 gene has a sequence of TAA repeats immediately following the poly C sequence, which are hypermutable and could offer an on-off mechanism for the expression of the gene, and changes of the repeat no. of the both tracts contribute to the variation of the fuct2 genotype in different strains. The invention further provides a method to measure the enzymic activity and acceptor specificity of .alpha.1,2-fucosyltransferase. The invention also relates to .alpha.1,2-fucosyltransferase antibodies which have research and diagnostic utility in the development of assays to detect mammalian tumors.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 2
ACCESSION NUMBER: 2000:148527 CAPLUS
DOCUMENT NUMBER: 132:290436
TITLE: Cloning and characterization of the .alpha.(1,3/4) fucosyltransferase of **Helicobacter pylori**
AUTHOR(S): Rasko, David A.; Wang, Ge; Palcic, Monica M.; Taylor, Diane E.
CORPORATE SOURCE: Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, T6G 2H7, Can.
SOURCE: Journal of Biological Chemistry (2000), 275(7), 4988-4994
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The gastric pathogen **Helicobacter pylori** can express the histo

blood group antigens, which are on the surface of many human cells. Most *H. pylori* strains express the type II carbohydrates, **Lewis X** and **Y**, whereas a small population express the type I carbohydrates, **Lewis A** and **B**. The expression of **Lewis A** and **Lewis X**, as in the case of *H. pylori* strain UA948, requires the addn. of fucose in .alpha.1,4 and .alpha.1,3 linkages to type I or type II carbohydrate backbones, resp. This work describes the cloning and characterization of a single *H. pylori* **fucosyltransferase** (FucT) enzyme, which has the ability to transfer fucose to both of the aforementioned linkages in a manner similar to the human **fucosyltransferase V** (Fuc-TV). Two homologous copies of the fucT gene have been identified in each of the genomes sequenced. The characteristic adenosine and cytosine tracts in the amino terminus and repeated regions in the carboxyl terminus are present in the DNA encoding the two UA948fucT genes, but these genes also contain differences when compared with previously identified *H. pylori* fucTs. The UA948fucTa gene encodes an approx. 52-kDa protein contg. 475 amino acids, whereas UA948fucTb does not encode a full-length FucT protein. In vitro, UA948FucTa appears to add fucose with a greater than 5-fold preference for type II chains but still retains significant activity using type I acceptors. The addn. of the fucose to the type II carbohydrate acceptors, by UA948FucTa, does not appear to be affected by fucosylation at other sites on the carbohydrate acceptor, but the rate of fucose transfer is affected by terminal fucosylation of type I acceptors. Through mutational anal. we demonstrate that only FucTa is active in this *H. pylori* isolate and that inactivation of this enzyme eliminates expression of all Lewis antigens.

REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 3

ACCESSION NUMBER: 1999:651501 CAPLUS

DOCUMENT NUMBER: 132:1916

TITLE: Phase variation in *Helicobacter pylori* lipopolysaccharide due to changes in the lengths of Poly(C) tracts in .alpha.3-**fucosyltransferase** genes

AUTHOR(S): Appelmelk, Ben J.; Martin, Steve L.; Monteiro, Mario A.; Clayton, Chris A.; McColm, Andrew A.; Zheng, Pengyuan; Verboom, Theo; Maaskant, Janneke J.; Van den Eijnden, Dirk H.; Hokke, Cornelis H.; Perry, Malcolm B.; Vandenbroucke-Grauls, Christina M. J. E.; Kusters, Johannes G.

CORPORATE SOURCE: Departments of Medical Microbiology, Vrije Universiteit, Medical School, Amsterdam, 1081 BT, Neth.

SOURCE: Infection and Immunity (1999), 67(10), 5361-5366
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The lipopolysaccharide (LPS) of *Helicobacter pylori* expresses the **Lewis x** (Lex) and/or Ley antigen. It was shown previously that *H. pylori* LPS displays phase variation whereby an Lex-pos. strain yields variants with different LPS serotypes, for example, Lex plus Ley or nonfucosylated poly(lactosamine). *H. pylori* has two .alpha.3-**fucosyltransferase** genes that both contain poly(C) tracts. We now demonstrate that these tracts can shorten or lengthen randomly, which results in reversible frameshifting and inactivation of the gene products. We provide genetic and serol. evidence that this mechanism causes *H. pylori* LPS phase variation and demonstrate that the on or off status of .alpha.3-**fucosyltransferase** genes detcs. the LPS serotypes of phase variants and clin. isolates. The role of the .alpha.3-**fucosyltransferase** gene products in detg. the LPS serotype was

confirmed by structural-chem. anal. of .alpha.3-**fucosyltransferase** knockout mutants. The data also show that the two .alpha.3-**fucosyltransferase** genes code for enzymes with different fine specificities, and we propose the names futA and futB to designate the orthologs of the H. pylori 26695 .alpha.3-**fucosyltransferase** genes HP0379 and HP0651, resp. The data also show that the .alpha.3-fucosylation in H. pylori precedes .alpha.3-**fucosyltransferase**, an order of events opposite to that which prevails in mammals. Finally, the data provide an understanding at the mol. level of the mechanisms underlying LPS diversity in H. pylori, which may play an important role in adaptation to the host.

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 9 OF 15 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 1999:890888 SCISEARCH

THE GENUINE ARTICLE: 255GA

TITLE: Novel **Helicobacter pylori** alpha 1,2-**fucosyltransferase**, a key enzyme in the synthesis of Lewis antigens

AUTHOR: Wang G; Boulton P G; Chan N W C; Palcic M M; Taylor D E (Reprint)

CORPORATE SOURCE: UNIV ALBERTA, DEPT MED MICROBIOL, EDMONTON, AB T6G 2H7, CANADA (Reprint); UNIV ALBERTA, DEPT MED MICROBIOL, EDMONTON, AB T6G 2H7, CANADA; UNIV ALBERTA, DEPT IMMUNOL, EDMONTON, AB T6G 2H7, CANADA; UNIV ALBERTA, DEPT CHEM, EDMONTON, AB T6G 2H7, CANADA

COUNTRY OF AUTHOR: CANADA

SOURCE: MICROBIOLOGY-UK, (NOV 1999) Vol. 145, Part 11, pp. 3245-3253.

Publisher: SOC GENERAL MICROBIOLOGY, MARLBOROUGH HOUSE, BASINGSTOKE RD, SPENCERS WOODS, READING RG7 1AE, BERKS, ENGLAND.

ISSN: 1350-0872.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 33

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Helicobacter pylori** lipopolysaccharides (LPS) contain complex carbohydrates known as Lewis antigens which may contribute to the pathogenesis and adaptation of the bacterium. Involved in the biosynthesis of Lewis antigens is an alpha 1,2-**fucosyltransferase** (FucT) that adds fucose to the terminal beta Gal unit of the O-chain of LPS. Recently, the H. pylori (Hp) alpha 1,2-fucT-encoding gene (fucT2) was cloned and analysed in detail. However, due to the low level of expression and instability of the protein, its enzymic activity was not demonstrated. In this study, the Hp fucT2 gene was successfully overexpressed in *Escherichia coli*. Sufficient amounts of the protein were obtained which revealed alpha 1,2-**fucosyltransferase** activity to be associated with the protein. A series of substrates were chosen to examine the acceptor specificity of Hp alpha 1,2-fucT, and the enzyme reaction products were identified by capillary electrophoresis. In contrast to the normal mammalian alpha 1,2-FucT (H or Se enzyme), Hp alpha 1,2-FucT prefers to use **Lewis X** [beta Gal1-4(alpha Fuc1-3)beta GlcNAc] rather than LacNAc [beta Gal1-4 beta GlcNAc] as a substrate, suggesting that H. pylori uses a novel pathway (via **Lewis X**) to synthesize Lewis Y. Hp alpha 1,2-FucT also acts on type 1 acceptor [beta Gal1-3 beta GlcNAc] and Lewis a [beta Gal1-3(alpha Fuc1-4)beta GlcNAc], which provides H. pylori with the potential to synthesize H type 1 and Lewis b epitopes. The ability to transfer fucose to a monofucosylated substrate (**Lewis X** or Lewis a) makes Hp alpha 1,2-FucT distinct from normal mammalian alpha 1,2-FucT.